GPEN 2006

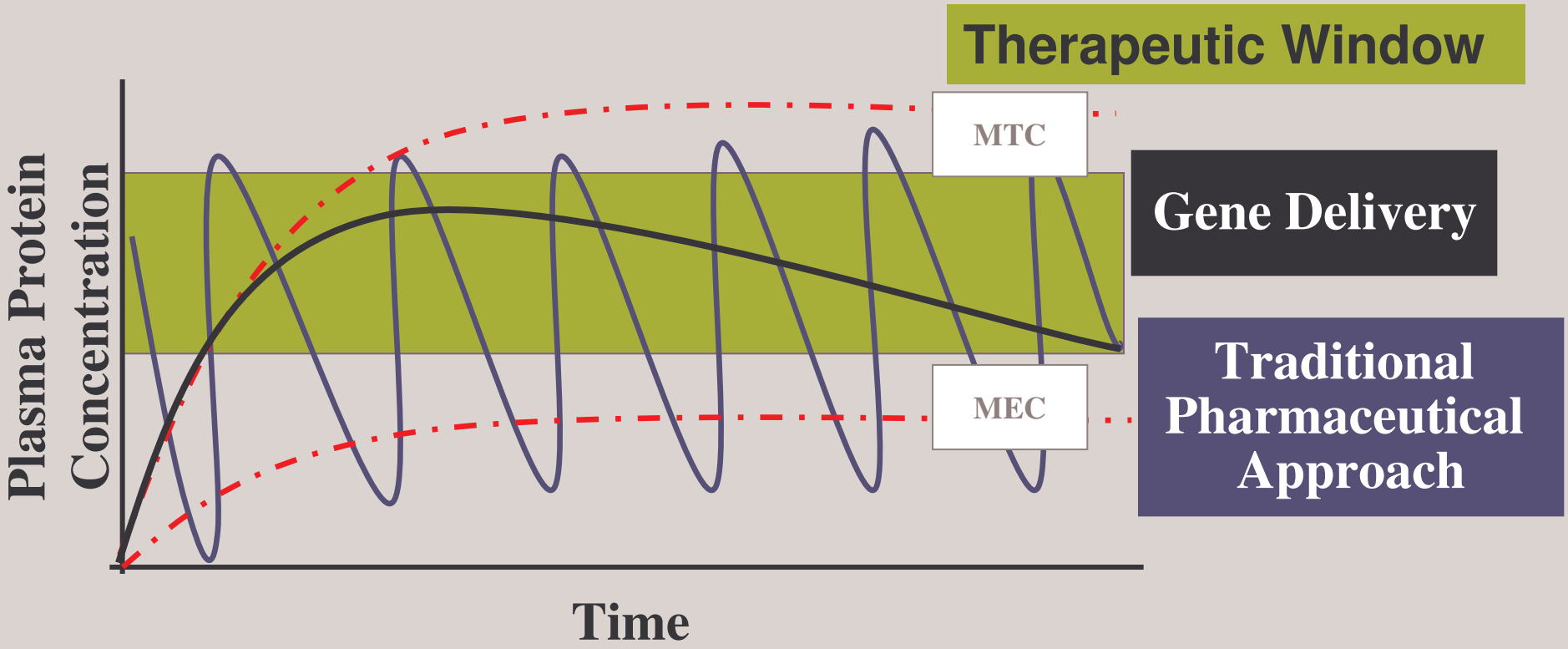
The University of Utah

Reducible Poly(amido ethylenimine)s for Nucleic Acid Delivery

L.V. Christensen[1], C.-W. Chang[1], J.H. Jeong[1], Z. Zhong[2], J. Feijen[2], and S.W. Kim[1]

[1]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah.
[2]Department of Polymer Chemistry and Biomaterials and Institute for Biomedical Technology (BMTI), University of Twente, The Netherlands.

Why Gene Therapy?

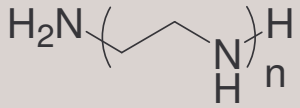


Adapted from Clackson, *Gene Ther.*, 7,120 (2000).

Vector Systems for Nucleic Acid Delivery

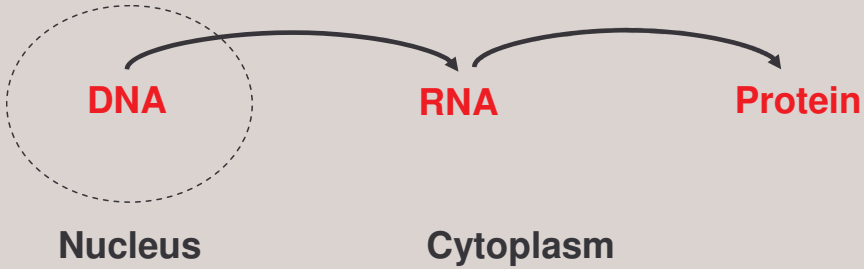
- **Ability to deliver a diverse range of genetic material**
  → RNAi, shRNA, oligos, pDNA

- **Chemistry allows for modification/targeting**
  → specificity, hydrophobic moieties, pegylation



- **High transfection efficiency → PEI based system??**

- **Minimal toxicity due to direct exposure or immune response → biodegradable carrier systems?? PEG conjugation??**



Biodegradable Polymers

Biodegradable Poly(ethylene glycol)-*co*-poly(L-lysine)-*g*-histidine Multiblock Copolymers for Nonviral Gene Delivery

Macromolecules **2004**, *37*, 1903−1916

A versatile family of degradable non-viral gene carriers based on hyperbranched poly(ester amine)s

Journal of Controlled Release 109 (2005) 317–329

Biodegradable Polymeric Vectors for Gene Delivery to Human Endothelial Cells

Bioconjugate Chem. **2006**, *17*, 1162–1169

Polyethylenimine with acid-labile linkages as a biodegradable gene carrier

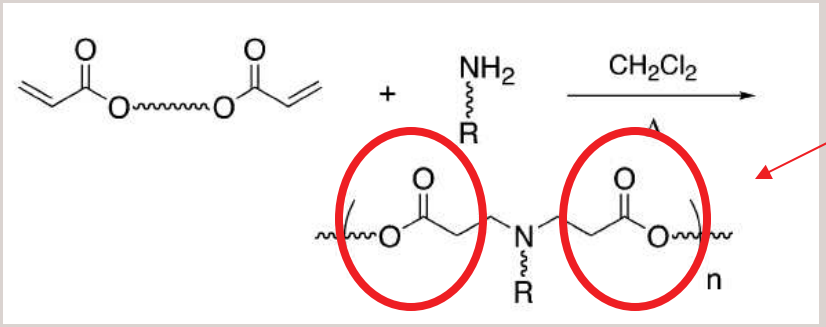
Journal of Controlled Release 103 (2005) 209–219

Degradable gene carriers based on oligomerized polyamines

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES XXX (2006) XXX–XXX

Parallel Synthesis and Biophysical Characterization of a Degradable Polymer Library for Gene Delivery

J. AM. CHEM. SOC. 2003, *125*, 5316−5323



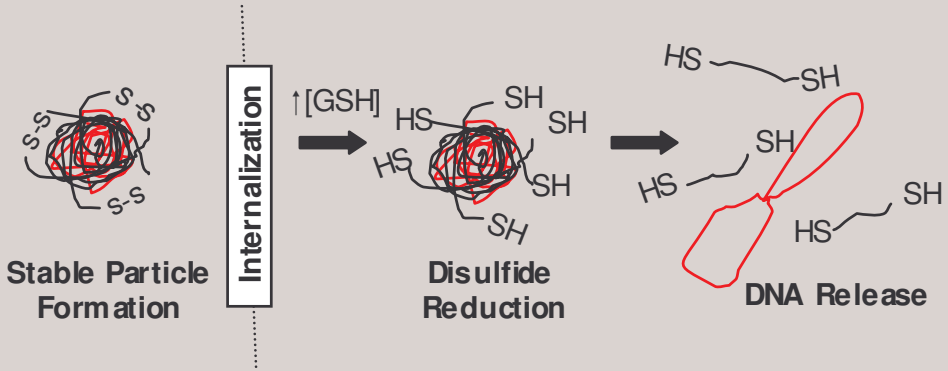
Hydrolytically unstable

Glutathione Reduction Inside the Cell

- Intracellular [GSH] is 50 - 1000 fold higher than extracellular space

- Bacterial toxins cholera and diphtheria take advantage of redox potential during translocation into host cells

- Disulfide bioconjugation has been popular in a variety of cellular drug delivery systems → enhanced cytosolic delivery, improved pharmacokinetics, and increased stability

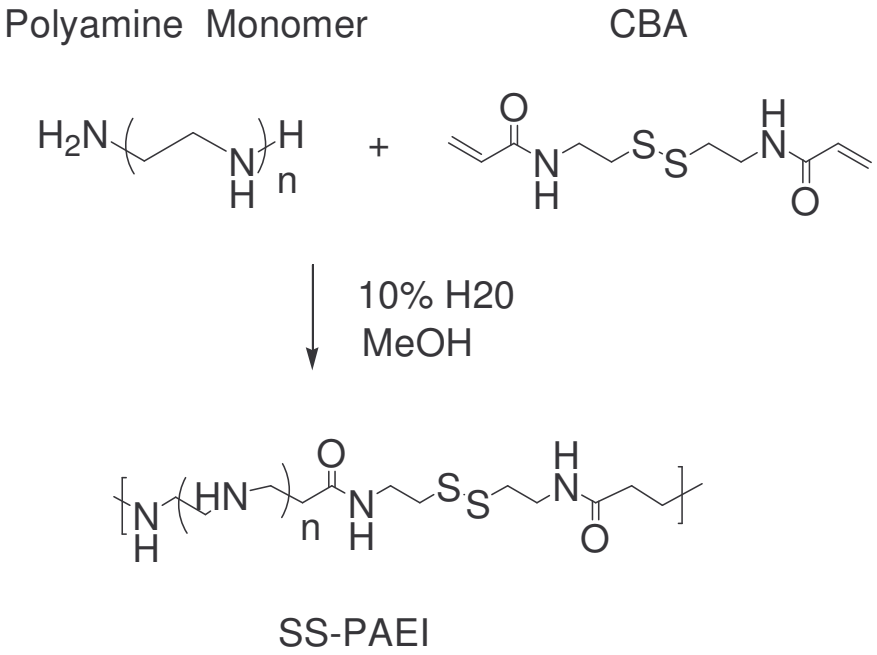


Adv Drug Del Rev (2003) 55; 199-215.

Reducible Poly(amido ethylenimine)s (SS-PAEI)s

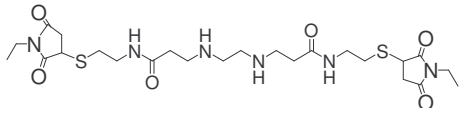
- **Polymers synthesized at 50º C under Argon and purified using MWCO 1000 high pressure dialysis system**

- **3 new polymers synthesized and characterized via 1H-NMR, GPC, acid-base titration, and LC-MS**

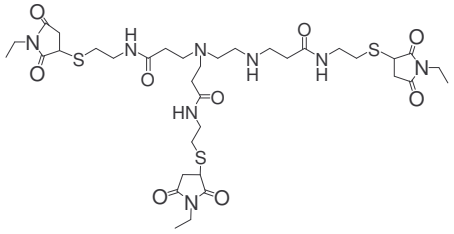


Characterization of SS-PAEIs

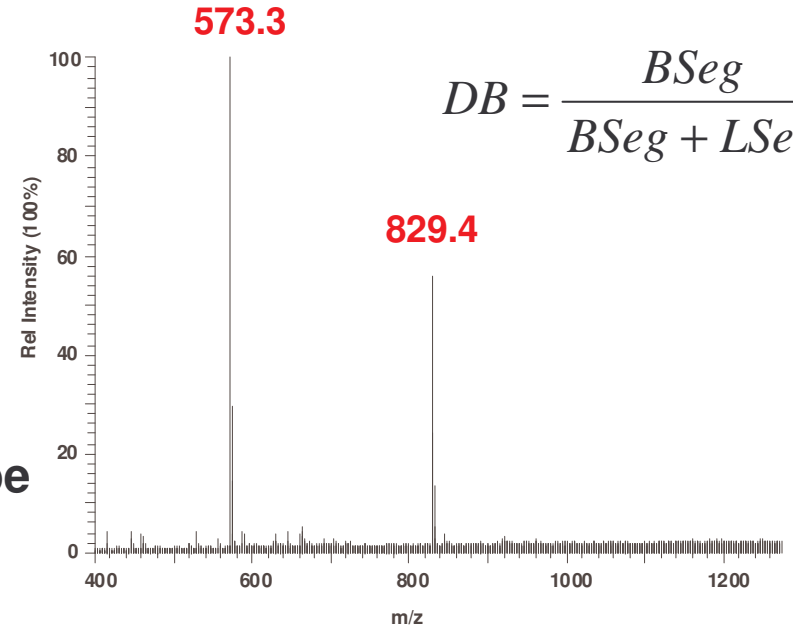
Polymer	Reaction Time	Mn (kDa)	Mw (kDa)	Mw/Mn	Titration (μmol HCl)	% Protonated Nitrogens	Degree of Branching
poly(EDA/CBA)	48 h	1.6	2.0	1.26	8.2	21.9	0.37
poly(DETA/CBA)	18 h	3.3	4.8	1.47	5.0	10.1	0.72
poly(TETA/CBA)	16 h	2.3	3.4	1.45	8.5	14.4	0.64
bPEI 25k	--	6.5	9.5	1.47	18.8	13.5	--
LPEI 25k	--	7.2	13.1	1.82	16.0	11.5	--



Mass: 572.3



Mass: 828.3

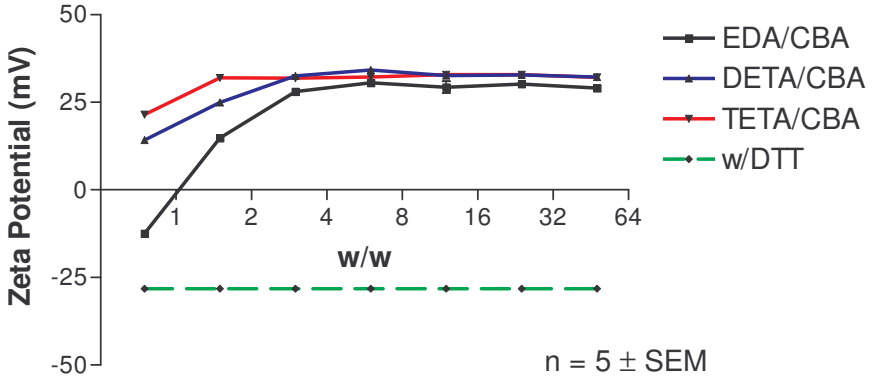


$$DB = \frac{BSeg}{BSeg + LSeg}$$

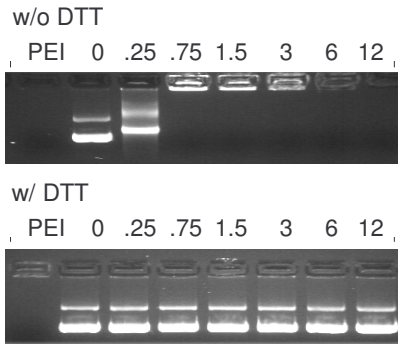
- **Buffering capacity seems to be correlated with degree of branching**

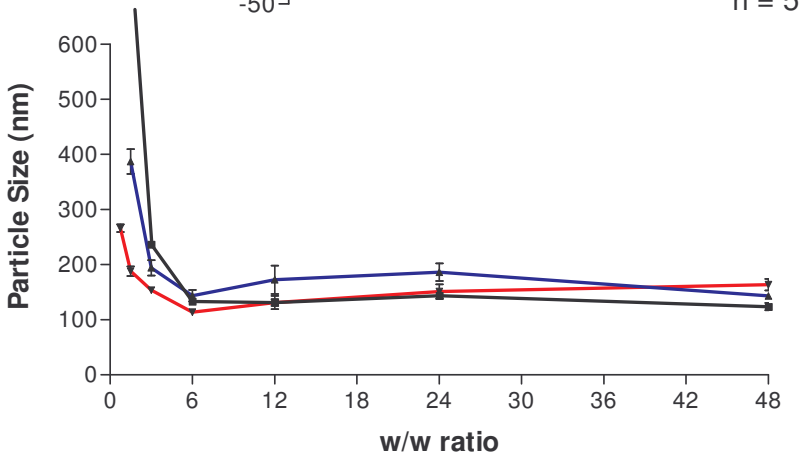
Polymer/pDNA Complex Characterization

- **Form stable nanoparticles < 200 nm**
- **There does not seem to be any interaction with pDNA when polymer is reduced**



n = 5 ± SEM



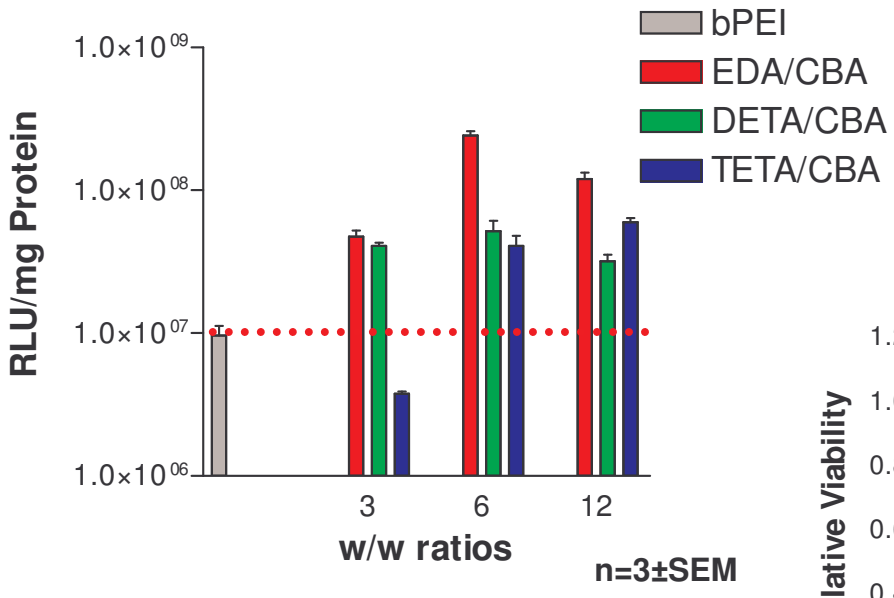


n = 3 ± SEM

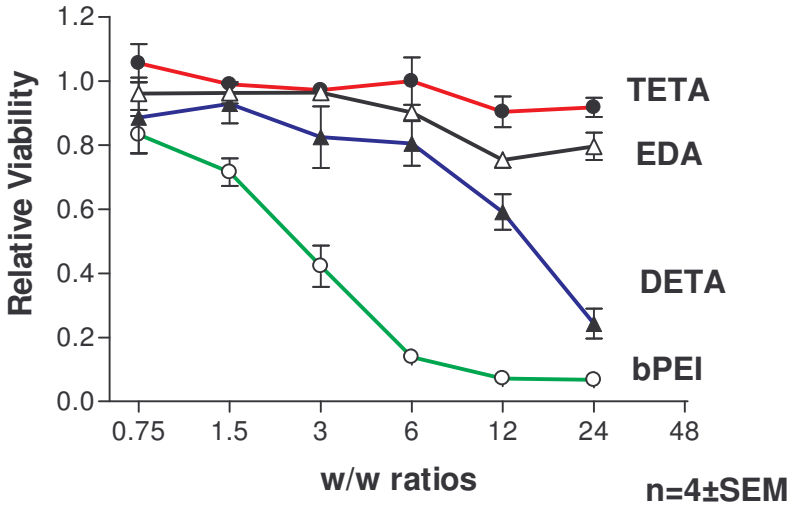
DTT: disulfide reducing agent

Bioconjugate Chemistry (2006) 17; 1233-1240.

Transfection and Cell Toxicity on BAECs



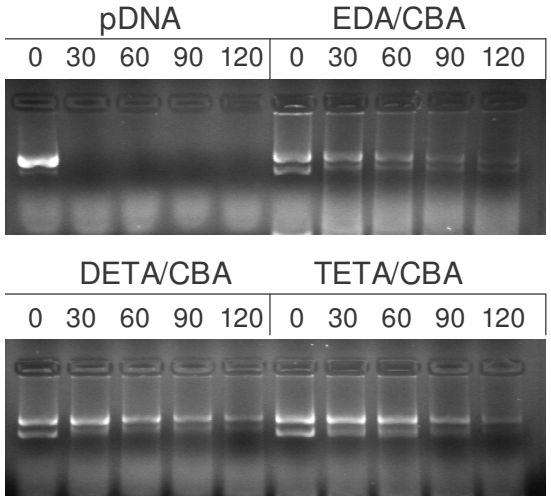
- **Luc analysis done 48 hr post transfection**
- **MTT assay performed 24 hr post transfection**



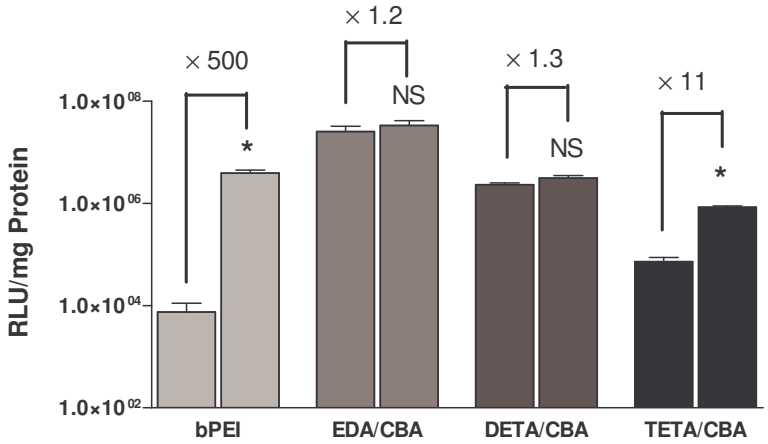
- **Transfections produced similar results for fibroblasts (NIH3T3), endothelial cells (BAEC) and smooth muscle cells (A7R5)**

Serum Interaction and DNA Protection

DNAase I incubation



w vs w/o serum in media

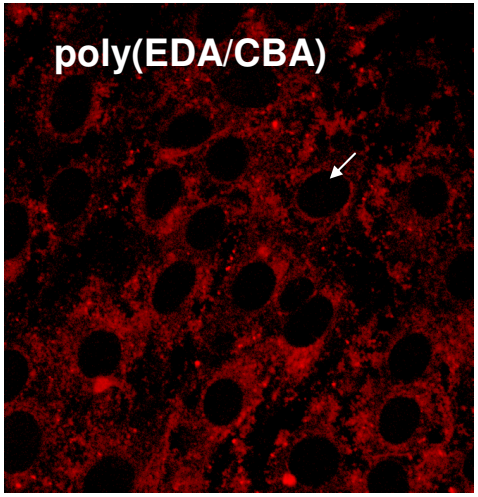


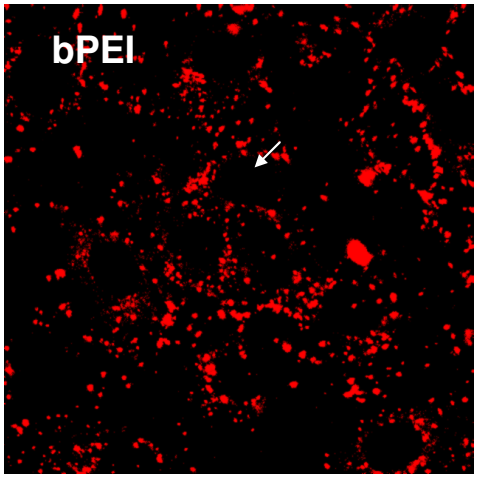
n=3±SEM, *p<0.05

- PEI has significantly higher amount of serum interactions
- Protect against DNAse degradation

Bioconjugate Chemistry (2006) 17; 1233-1240.

Confocal Microscopy on EMA Distribution



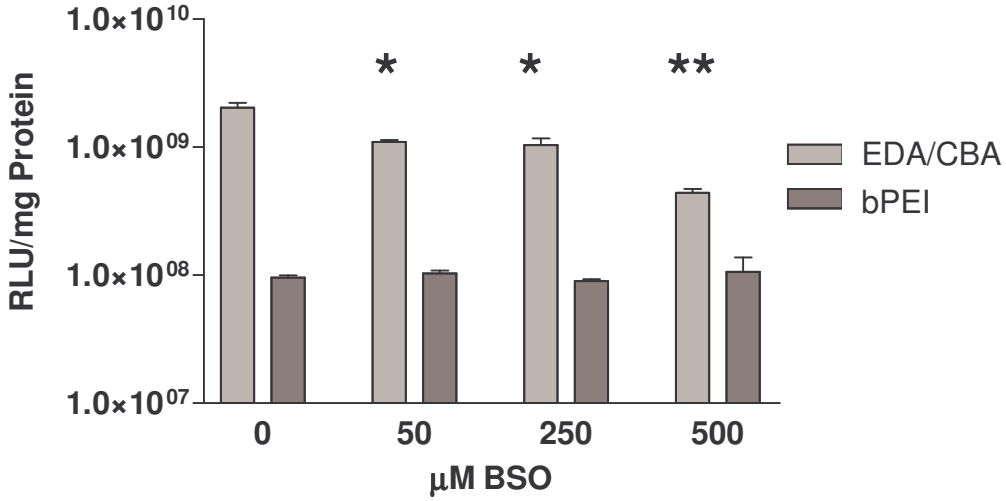


- Ethidium monoazide (EMA) labeled pDNA was used for transfections on NIH 3T3
- Reducible polymer displays more dispersed fluorescence after 6 hr compared to bPEI
- Earlier time points did not reveal such a difference in fluorescence

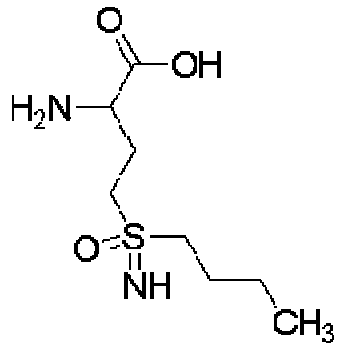
→ Due to reducible disulfide bonds?

Bioconjugate Chemistry (2006) 17; 1233-1240.

Effect on the Presence of GSH Inhibitor



n=3±SEM, *p<0.01 **p<0.001



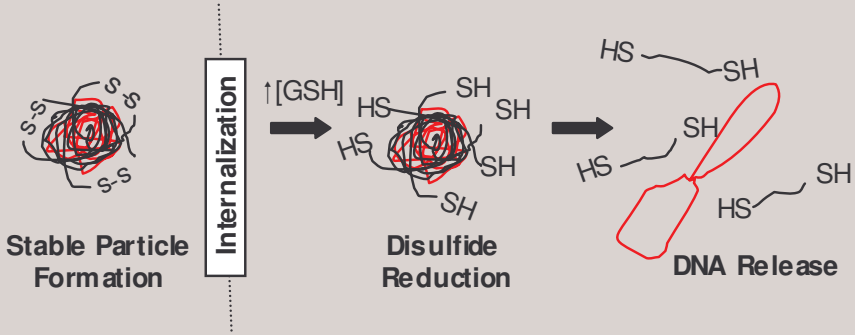
DL-Buthionine Sulfoxamine (BSO)

- BSO decreases intracellular GSH
- Gene expression dependent upon GSH levels

Molecular Therapy (2006) *in submission*.

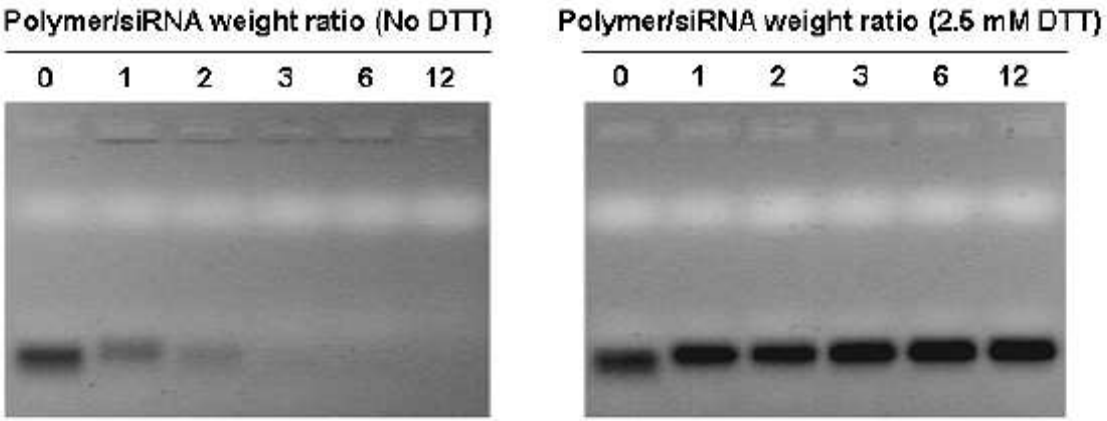
Conclusions for DNA Delivery

- New class of biodegradable polymer synthesized and characterized
- Polymers interact with pDNA to form stable complexes able to transfect pDNA
- Polymers show higher expression than control at lower toxicity
- Reducible polymer mediated transfection is dependent upon GSH



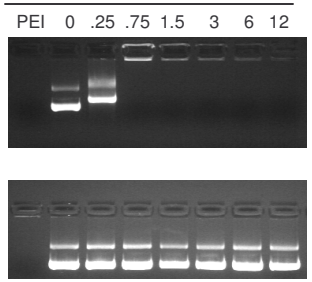
- What about siRNA delivery??
- 200 bp vs 5 kbp
- Targeting the cytoplasm instead the nucleus

Gel Electrophoresis of Polymer/siRNA



- **Gel electrophoresis shows similar complexing w/w**
- **Presence of a reducing agent prevents interactions at all w/w looked at**

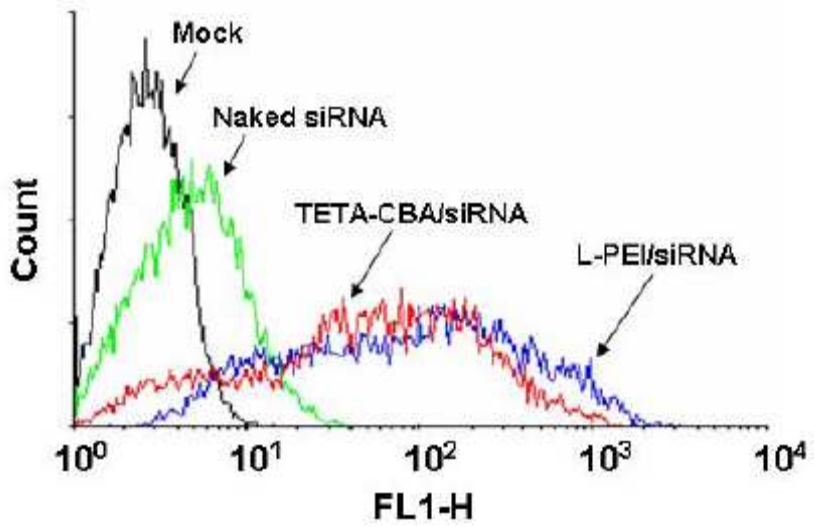
Complexing w/DNA



DTT: disulfide reducing agent

Biomaterials (2006) *in submission*.

FACS Analysis of FITC-siRNA Delivery

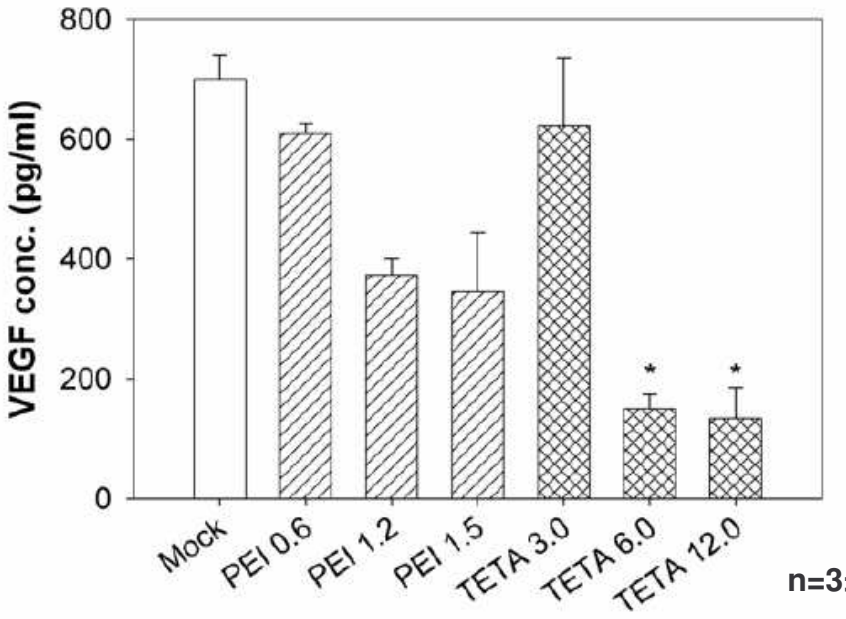


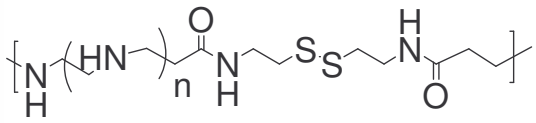
- **FITC labeled siRNA showed similar levels of cellular localization for both polymer carriers looked at in human prostate carcinoma cells (PC-3)**

- **Polymer mediated delivery showed higher localization than naked delivery**

Biomaterials (2006) ***in submission*****.**

VEGF Suppression *In Vitro*



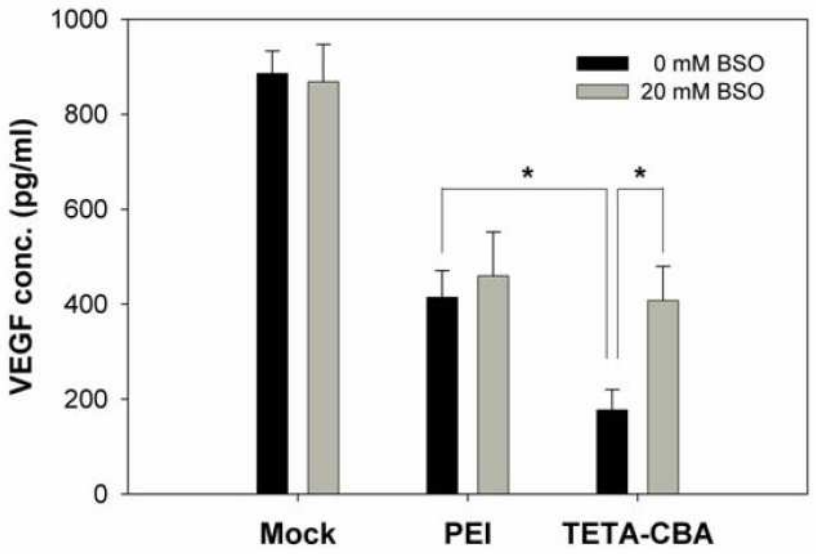


n=3±SD, *p< 0.01

- RNAi-induced expression of VEGF expression on PC-3 cells
- TETA-CBA mediated delivery showed statistically higher reduction of VEGF

Biomaterials (2006) *in submission*.

Effect on the Presence of BSO



n=3±SD, *p< 0.01

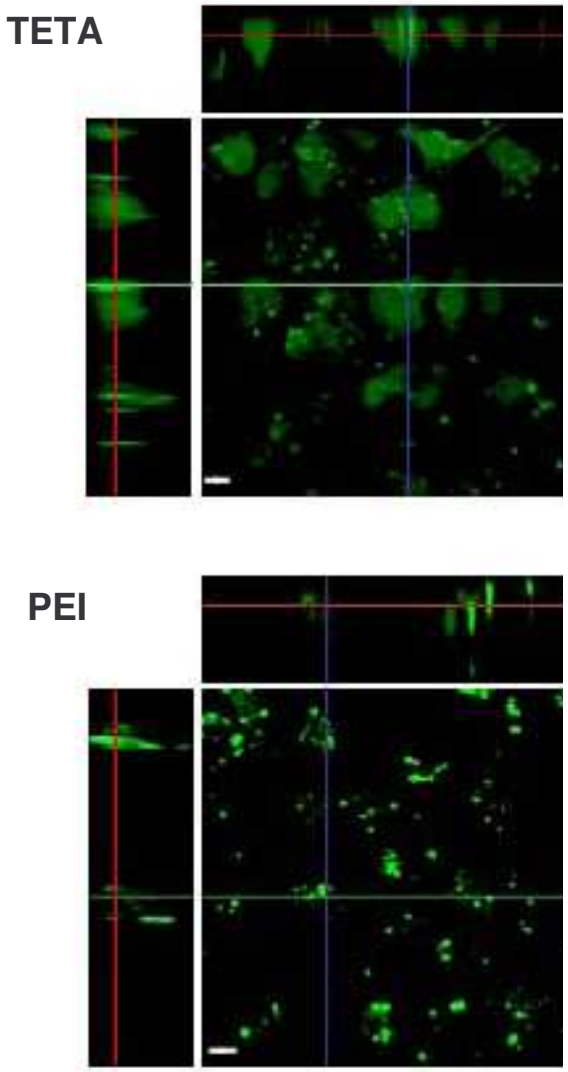
- Effect of BSO on the siRNA-mediated expression of VEGF on PC-3 cells for PEI and TETA-CBA at 1.2:1 and 6:1 w/w respectvely

- Delivery of reducible polymer mediated siRNA again seems GSH dependent

Biomaterials (2006) *in submission*.

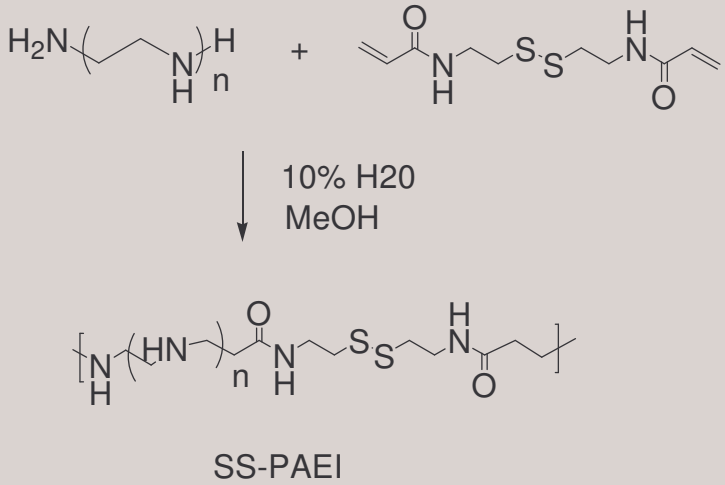
Confocal Microscopy

- FITC labeled siRNA was used for delivery to PC-3 cells
- Reducible polymer mediated delivery displays more dispersed fluorescence after 5 hr compared to bPEI
- Lower magnification demonstrates the same trend throughout the cellular population



Biomaterials (2006) *in submission*.

Conclusions Using siRNA



SS-PAEI

- **SS-PAEIs are a new type of biodegradable carrier that can be used for efficient intracellular delivery of a broad range of nucleic acids**
- **SS-PAEIs successfully interact with siRNA long enough to deliver a significant effect in vitro**
- **~90% knockdown of VEGF was seen for siRNA delivery compared to only ~65% for a PEI control**
- **Experiments showed a dependence upon GSH inside the cell**
- **Confocal microscopy experiments detailed the difference in delivery between reducible and nonreducible cationic polymers**
- **Results comparable to pDNA delivery**

Acknowledgements

- Prof. Sung Wan Kim
- Dr. Jihoon Jeong
- Chien-Wen Chang
- Dr. James Yockman
- SWK lab members

THE UNIVERSITY OF UTAH

- Prof. Jan Feijen
- Dr. Zhiyuan Zhong
- Dr. Pete Dijkstra
- Prof. J.F.J. Engbersen
- PBM group members

University of Twente
Enschede – The Netherlands

Committee

- Dr. Steve Kern
- Dr. James Herron
- Dr. Donald Blumenthal
- Dr. David Bull

- GPC analysis -- Xulin Jiang & Prof. Wim E. Hennink at Utrecht University Dept. of Pharmaceutics
- LC-MS assistance – Dr. Krishna Parsawar at the University of Utah Cores Facility
- Funded by NIH grant HL 65477